REMARKS

Claims 1-43 and 52 are pending and have been rejected. Claims 18-43 and 52 have been canceled and claims 1-17 have been amended to recite "a complex comprising a negatively-charged organic complex and a charged antigen, where (A) said organic complex and antigen are electrostatically associated and (B) said complex generates a CTL response when administered to a mammal, as supported by the specification at page 11, lines 5-7, page 19, line 11, and page 20, lines 10-17. Claims 1-43 and 52 remain in the case.

Double Patenting Rejection

Under the doctrine of obviousness-type double patenting, the examiner has provisionally rejected claims 1-43 and 52 over claims 1-32 and 35-47 of U.S. application serial No. 09/714438. As previously noted, applicants do not acquiesce to the stated rationale for this rejection. The issue is, as stated, provisional, and only properly considered in the context of the second application to reach the stage of allowance. Once allowable subject matter is indicated in this application, applicants will revisit the issue of obviousness-type double patenting, and are amenable to employing a terminal disclaimer should that be required.

Section 112 Rejection

Claims 18-43 and 52 are rejected under the first paragraph of Section 112, the examiner again contending that the specification does not support "any positively charged protein and any negatively charged adjuvant." While not acquiescing in the basis for this rejection, applicants are canceling claims 18-43 and 52 in this application, subject to applicants right to pursue these claims in a divisional application. This obviates the outstanding rejection under the first paragraph of Section 112.

Section 102(b) and 103(a) Rejection

Claims 1-8 and 12-14 are rejected over Nakanishi *et al.*, with the examiner asserting anticipation and obviousness in the alternative. Claim 1 has been amended to recite that the complex comprises a ***negatively-charged*** organic complex and a charged antigen. Nakanishi *et al.* teach that *only positively charged liposomes* can induce CTL.

(For example, see Figure 2, page 795 at column 1, paragraph 1, and the author's conclusions at page 796, column 2.) If anything, therefore, the cited Nakanishi document actually teaches away from the claimed invention.

The examiner takes the position that Nakanishi *et al.* made negatively-charged vesicles, all of which contain phosphatidylcholine and have been enhanced to become more negatively charged by the addition of phosphatidic acid. While the examiner admits that Nakanishi does not explicitly teach that the antigens have a positive charge and are electrostatically associated with the negatively-charged vesicle, she urges that both of these features would have been inherent, because "the negatively charged end of [phosphatidylcholine] would be found in the interior of the vesicle, which associates directly with the protein antigen." She further alleges that there would be an electrostatic interaction between the negatively-charged phosphatidylcholine of the vesicles and the antigen "since some amino acids inherently have a positive charge, and would be naturally attracted to the negative charge of the phosphatidylcholine, creating an electrostatic association between the antigen and the vesicle in some degree."

A careful reading of Nakanishi *et al.*, however, reveals the flaws in this reasoning. In the first instance, the conclusion that "the negatively charged end of [phosphatidylcholine] would be found in the interior of the vesicle" evidences a misunderstanding of phospholipid, and hence liposome, structure. The examiner was correct in the Action dated 23 May 2001, when she stated that phosphatidylcholine is an amphipathic molecule. The allegation that a negatively-charged end of this molecule is found in the interior of a liposome is incorrect, however. Liposomes consist of cholesterol, and one or more phospholipids. In their simplest manifestation, they are a bilayer membrane with the headgroup exposed at the internal and external surface. Each layer of the bilayer is aligned so that the hydrophobic sides abut and the hydrophilic sides are on the outside. A phospholipid consists of two hydrophobic chains, a phosphate group and a head group. The phosphate group carries a negative charge, while the head group may be positively-charged or neutral. Phosphatidylcholine (PC) has *both* a positive (choline) and negative (phosphate) entity exposed at the internal and external surfaces of the membrane, resulting in an overall neutral charge, *i.e.*, the liposome will be neutral at *both* its internal and external surface. Phosphatidic acid (PA)

lacks a head group and so is overall negative. In either case, the hydrophobic chains point toward the hydrophobic side of each layer, and the phosphate and head group point toward to the hydrophilic, *i.e.*, external, side of the layer. Between each bilayer there exists a film of liquid. The liquid necessarily contacts the hydrophilic sides of the bilayers, and only the external surface of the outermost bilayer membrane actually is exposed to the external environment.

Nakanishi *et al.* used conventional techniques to combine antigen and liposomes. According to such techniques, loading of liposomes is achieved by high pressure agitation, by vortexing or otherwise mixing a lipid film with an aqueous solution of antigen, so that fluid from the environment external to the lipid can be transferred to the internal spaces of the liposome, *i.e.*, the liquid-filled spaces between each shell. Where the external environment comprises antigen, disruption of the bilayer membranes in order to facilitate transfer of the external environment fluid to the internal layer necessarily will result also in the transfer of the antigen which is suspended in the external fluid. Subsequent to the disruption, the lipid bilayer membranes will automatically reform. The procedure is termed "loading" of the liposome. It is an extremely inefficient process, and any one event of liposome disruption and reforming results in the internalization of only a very small percentage of the external fluid. In order to achieve equilibration between the concentration of antigen in the external fluid and the internal fluid layers, multiple events of liposome membrane disruption and reforming must occur. At the point of equilibration, however, only a very small fraction of the external fluid will be trapped between the lipid bilayers. Indeed, the ratio of the internal "trapped" volume to the external "free" volume is generally of the order of only a few percent.

Conventional loading of liposomes as disclosed in Nakanishi clearly is an inefficient mechanical procedure which ***physically traps*** antigen by breaking and then reforming a physical barrier, the concentric bilayer membranes of the liposome. Prior to the present invention, there was no suggestion that this inefficiency could be overcome by selecting lipids for preparing liposomes which had a charge suitable for binding the antigen of choice to be loaded, an "electrostatic interaction" as recited in applicants' claims.

There is no basis for assuming any sort of electrostatic interaction in Nakanishi. Nakanishi uses only two antigens, chicken egg albumin (OVA) and beta-galactosidase. Chicken egg albumin has a pI of 4.9, and beta-galactosidase has a pI of 4.6. Assuming that the antigen and MLV are co-dispersed at 7.6 (the pH stated for the control), both antigens would be negatively charged in Nakanishi. Nakanishi purports to make combinations of these two negatively-charged antigens with negatively-charged, positively-charged and neutral liposomes. This conclusively shows that the interaction in Nakanishi is not an electrostatic interaction. Nakanishi achieve loading irrespective of whether the charge of the liposome is negative, positive or neutral, a clear indication that the association between the antigen and liposome is something other than electrostatic.

It is worth noting in this regard that Nakanishi *et al.* focus on the interaction between liposomes and macrophages, ***not*** the interaction between liposomes and antigen. Nakanishi *et al.* in Figure 4 show that all liposomes, regardless of charge, are able to enhance antibody response. Conversely, Nakanishi *et al.* show in Figure 2 that only positively-charged liposomes are able to induce CTL responses. This was attributed to the fact that only positively-charged liposomes were able effectively to bind to the surface of murine macrophages. The fact that the positively-charged liposomes were able to bind to macrophages suggest that these groups had not interacted with the antigen. Rather, this confirms that antigen loading was achieved via physical, non-electrostatic means which left the charged groups on the external surface of the liposome available for binding to the macrophages. Thus, Nakanishi *et al.* do not teach or suggest the combination of charged liposomes with oppositely-charged antigens to improve association between the two. Accordingly, the pending rejections for anticipation and obviousness based on Nakanishi *et al.* must be seen as ill-founded and warranting withdrawal.

Claims 9-11 and 15-17 are rejected under Section 103(a) based on Nakanishi *et al.* in view of Barr *et al.* The comments above with respect to Nakanishi are *et al.* incorporated here. As to Barr *et al.*, applicants again wishes to clarify the role of saponins and phospholipids within ISCOMs. Interaction between saponin and cholesterol causes formation of a ring structure, about 10 nm in diameter, that, in the presence of lipids (preferably phospholipids) and under fairly broad stoichiometric constraints, self-

assemble into 40 nm, open cage-like structures. These complexes have two important properties. They contain *Quillaia* saponins, which are powerful immunomodulators, and they comprise hydrophobic regions, where hydrophobic antigens can be bound. Phospholipids are ***not*** variants of saponins. Rather, phospholipids are ***normal*** components of cell membranes and therefore necessarily ***lack*** immunological activity. Saponins, on the other hand, disrupt cell membranes, and one subset, *Quillaia* saponins, have strong immunomodulating activities, as would be expected for an adjuvant. Thus, a saponin is an adjuvant and a phospholipid is ***not*** an adjuvant. It is, therefore, improper to combine Nakanishi *et al.* and Barr *et al.* since these authors were investigating different aspects of an immunological response. Nakanishi *et al.* were investigating the role of liposomes in the context of their interaction with macrophages and the induction of immune responses. In this context the liposomes provided a vehicle for ***delivering*** antigen, but did not, and cannot, function as an immunomodulatory adjuvant. Barr *et al.*, in contrast, relate to the ISCOMS, which structures comprise an adjuvant. An adjuvant is a substance which acts to enhance an immune response to an antigen with which it is administered.

The alleged nexus between Nakanishi *et al.* and Barr *et al.* is the lipids of the latter and the phospholipids of the former ("Barr *et al* review different ISCOMs, and name saponin, lipid A, and phospholipids (taught by Nakanishi *et al.*) as obvious variants to one another"). Yet neither the lipids of ISCOMS nor the phospholipids of liposomes are adjuvants. Only the saponin component of the ISCOM is an adjuvant. The examiner thus compares molecules which exhibit completely unrelated and distinct immunological functions in the context of their respective references to arrive at a conclusion that they are "obvious variants." This is clearly improper. Reconsideration and withdrawal of the rejection based on the combination of Nakanishi *et al.* and Barr *et al.* is respectfully requested.

Applicants note that the current Action included Raw Sequence Listing Error Summary. A corrected Sequence Listing is being prepared and will be forwarded as soon as received.

Based on the foregoing, applicants submit that the present claims are in allowable condition, and favorable reconsideration of the application therefore is requested. The examiner is invited to contact the undersigned, should there be any other issues that may require consideration.

Respectfully submitted,

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Versions with Markings to Show Changes Made

Please cancel claims 18-43 and 52 and amend the remaining claims as follows:

1. (Twice Amended) [An immunogenic] A complex comprising a negatively-charged organic complex and a charged antigen, [which] where (A) said organic complex and antigen are electrostatically associated and (B) said complex generates a CTL response when administered to a mammal.

2. (Twice Amended) The [immunogenic] complex according to claim 1 wherein [said charged organic complex is negatively charged and] said antigen is positively charged.

3. (Amended) The [immunogenic] complex according to claim 2 wherein said antigen is a protein or derivative or equivalent thereof.

4. (Twice Amended) The [immunogenic] complex according to claim 2 wherein said negatively-charged organic complex is an adjuvant or derivative or equivalent thereof.

5. (Twice Amended) The [immunogenic] complex according to claim 2 wherein said antigen is a protein or derivative or equivalent thereof and said negatively-charged organic complex is an adjuvant or derivative or equivalent thereof.

6 . (Twice Amended) The [immunogenic] complex according to claim 5 wherein said adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge.

7. (Twice Amended) The [immunogenic] complex according to claim 5 wherein said protein is a naturally positively charged protein which has been modified to increase the degree of its positive charge.

8. (Twice Amended) The [immunogenic] complex according to claim 5 wherein said adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge and said protein is a naturally

positively charged protein which has been modified to increase the degree of its positive charge.

9. (Twice Amended) The [immunogenic] complex according to claim 5 wherein said adjuvant comprises a saponin.

10. (Twice Amended) The [immunogenic] complex according to claim 5 wherein said adjuvant is a saponin complex.

11. (Amended) The [immunogenic] complex according to claim 10 wherein said saponin complex is ISCOMATRIX™.

12. (Amended) The [immunogenic] complex according to claim 5 wherein said adjuvant is comprises a phospholipid.

13. (Amended) The [immunogenic] complex according to claim 12 wherein said phospholipid is a phosphoglyceride.

14. (Amended) The [immunogenic] complex according to claim 13 wherein the phosphoglyceride is selected from the group consisting of phosphatidyl inositol, phosphatidyl glycerol, phosphatidic acid and cardiolipin.

15. (Amended) The [immunogenic] complex according to claim12 wherein said phospholipid is lipid A.

16. (Twice Amended) The [immunogenic] complex according to claim 15 wherein the lipid A is selected from the group consisting of diphosphoryl lipid A and monophosphoryl lipid A.

17. (Twice Amended) The [immunogenic] complex according to claim 1 wherein said complex induces a cytotoxic T-lymphocyte response.